**ABSTRACT**

Rice is the staple food source of nutrients for both humans and livestock throughout the world. The major proteins in rice seeds are the storage proteins, glutelins and prolamins. Their mRNAs are localized at different subdomains of the endoplasmic reticulum (ER). Glutelin mRNAs are targeted to the cisternal ER, while prolamin mRNAs are targeted to the protein body ER (PB-ER) membranes that delimit intracisternal prolamin inclusions. The glup4 mutant, which causes glutelin mRNAs to be transported to the PB-ER instead of their normal destination on the cisternal ER, lacks the small GTPase, Rab5a. In order to find the underlying cause of this error in mRNA targeting, these studies were undertaken to identify an associated effector protein of Rab5a that specifies its role in RNA targeting. We used Escherichia coli to express GST-tagged-Rab5a positive and negative constructs. Rab5a-Q70L, the GTP fixed (active) form, was used as a positive control while Rab5a-EM960, which has mutation in the effector region (G45D), was used as a negative control. The purified GST-Rab5a proteins were attached to Glutathione-linked Sepharose resin to create an affinity chromatography column. These columns were used “fish out” the Rab5a associated effector proteins in rice developing seed extracts. The eluted proteins from the positive and negative Rab5a columns were directly compared by SDS polyacrylamide gel electrophoresis. Initial studies show no significant differences in the polypeptides eluted from these columns.

**RESULTS**

**Methods**

**Summary of Events**

1. **Growth**
   - Grow E. coli cells containing constructs in LB media until OD600 is around 0.5.
   - Add IPTG to final concentration of 0.1 mM to express GST-fused Rab5a proteins.

2. **De-husk and homogenize 7g of developing seeds in 3mL per 1g.**
   - Add 200ug of GST-Rab5a to the homogenate.
   - Centrifuge supernatant at 100 g for 1 min to remove large starch grain. Then add 2x volume of wash buffer A and centrifuge supernatant at 1000 rpm for 10 min to get pellet. Resuspend the pellet in wash buffer B containing 1% Triton X-100.

3. **Pellet:**
   - Resuspend the pellet in CSB buffer containing 1% Triton X-100.
   - Incubate with GST-fused Rab5a protein for 4 hours.
   - Check the starting material, resin, and flow through for the column condition by SDS-PAGE.

4. **Incubate supernatant with Glutathione Sepharose resin for 2 hours to create mutant Rab5a affinity columns.**

5. **Wash with buffer B until A280 is near zero.**

6. **Wash with buffer A until A280 is near zero.**

7. **Collect flow through sample.**

8. **Add 0.5 mL Elute buffer (A2F) and collect eluant as sample.**

9. **Centrifuge to collect Eluted pellet and resuspended in lysis buffer, followed by cell disruption by microfluidizer.**

10. **Then centrifuge supernatant at 3000 rpm for 10 min to get pellet. Resuspend the pellet in CSB buffer containing 1% Triton X-100.**

11. **To determine effector protein, cut out specific band in Q70L elute sample and for tandem mass spectrometry analysis.**

**Purification of Rab5a Effector Responsible for Glutelin mRNA Location via Affinity Chromatography**

**Introduction**

Rice is a major staple food source as a source of dietary protein to both human and livestock (1, 2). The major storage proteins in rice (Oryza Sativa) are prolamine and glutelin, and are synthesized on the endoplasmic reticulum (ER). In rice endosperm, prolamin protein makes up 20-30% of total rice storage protein (1, 2). Prolamin mRNA is targeted to the PB-ER where it is translated and then it forms intracisternal inclusions genes within the ER lumen, a structure called PBI (3, 4, 5). On the other hand, glutelin makes up 60-80% of the protein content in endosperm (6, 2). Glutelin mRNAs are targeted to the cisternal ER. After they are synthesized as 57kDa glutelin precursor (proglutelin), they are transported to protein storage vacuoles (PSV) where they are cleaved into 30-36kDa acidic and 19-22kDa basic subunits, thus forming the PB-1 (3, 6, 8).

The glup4 mutant was induced by chemical mutagenesis using N-Methyl-N-Nitrosourea (MINU) (7, 9). This mutant is characterized by high proglutelin accumulation and the mis-localization of glutelin mRNA to the PB-ER rather than to the cisternal ER (4, 7, 8). From map-based cloning results, it was determined that this glup4 gene encodes a small GTPase Rab5a, a protein involved in membrane vesicular transport (3, 7, 8). The Rab family functions as regulators of distinct steps in membrane trafficking by recruiting specific effector proteins onto membranes in their active GTP fixed form (10). Through their effectors, Rab GTPases regulate vesicle formation, actin- and tubulin-dependent vesicle movement, and membrane fusion (10). Rab proteins are catalyzed by a GTP/GDP exchange factor (GEF) (10). In the Drosophila egg, VPS22 are 25, and 36 are part of an ESCRT-II complex that is associated with vesicle formation and bicoid mRNA localization (11). Therefore, rice Rab5a and VPS9 (GEF for Rab5a) may have the ability to participate in the mRNA targeting mechanism.

In order to learn more of the Rab5a associated trafficking process involved, we set out to find Rab5a-binding proteins from rice seed extract. By doing so, we expressed two Rab5a variants in E. coli. The Rab5a-Q70L mutant is the constitutively active form and EM960, a glut4 mutant line, contains a mutation (G45D) in its effector region, thus preventing its role in specifying membrane vesicular transport and targeting. We prepared affinity chromatography columns to probe for the unknown effector protein which is essential for membrane-vesicle associated RNA targeting (9). Results from SDS-PAGE of elution fractions showed specific bands in the positive control.

However, given the low protein content, we have to consider optimizing our experimental conditions.

**REFERENCES**


**Future Work**

- **Optimization of Rab5a affinity chromatography column.**
- **-Check buffer condition.**
- **-Tandem mass spec analysis of candidate effector protein bands if specific bands are presented in Q70L elute fraction.**
- **-Alternative methods: try pull-down assay or co-IP.**

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